ABSTRACTS:

Poster Presentations

Microtubules: Centrosomes and Centrioles

P1000/B1

PLK4 employs its cryptic polo box to scaffold the formation of pseudocentrosomes that accelerate acentriolar spindle assembly.

J. Xu[1], F. Meitinger[1,1,2,3], A. Desai[1,1,2], K. Oegema[1,1,2];
[1]University of California, San Diego, La Jolla, CA, [2]Ludwig Institute for Cancer Research, La Jolla, CA, [3]Okinawa Institute of Science and Technology, Onna, Japan

Segregation of replicated chromosomes during mitosis requires rapid assembly of a bipolar microtubule-based spindle. In metazoans, this is accomplished by a pair of centrosomes, composed of a centriolar core surrounded by a pericentriolar matrix, that catalyzes the assembly of spindle microtubules. In human cells that lack centrioles due to chemical inhibition of the centriole duplication kinase PLK4, spindle assembly occurs through slow self-organization of pericentriolar matrix containing foci, which takes about twice as long as in cells with centrosomes. Loss of TRIM37, a centrosome-regulating ubiquitin ligase, restores rapid bipolar spindle assembly in centrinone-treated acentriolar cells by enabling the formation of an array of foci that function as pseudocentrosomes. Pseudocentrosomes nucleate microtubules and robustly cluster to form two spindle poles. Pseudocentrosomes contain proteins proximal to PLK4 in the centrosome including CEP192, CEP152, CEP63 and the centriolar satellite protein PCM1, and are reminiscent of PLK4-containing foci required for naturally occuring acentriolar spindle assembly during the early mitotic divisions of mouse embryos. Using inducible gene knockouts in centrinone-treated *TRIM37Δ* cells, we found that PLK4 is the only centrosomal component of eight tested that was required for pseudocentrosome formation. The centrosomal scaffold protein CEP192, while dispensable for pseudocentrosome formation, was essential for their function; by contrast, the major pericentriolar matrix scaffold protein CDK5RAP2 was dispensable for both pseudocentrosome formation and function. To determine how PLK4 supports pseudocentrosome formation, we replaced endogenous PLK4 with mutants engineered to disrupt four of its known features: (a) kinase activity; (b) basic crater in the cryptic-polo box (CPB) domain that interfaces with centriolar receptors; (c) CPB homodimerization; and (d) oligomerization mediated by a C-terminal Polo Box domain. This analysis revealed that point mutations in the CPB basic crater prevented pseudocentrosome formation; by contrast, single or double depletion of the centriolar receptors CEP192 and CEP152 that bind to the basic crater did not. Thus, the PLK4 CPB basic crater is essential to form assemblies that recruit CEP192 and other components to build pseudocentrosomes that generate spindle microtubules. We speculate that PLK4-scaffolded pseudocentrosomes are analogous to structures that promote acentriolar spindle assembly in specific natural contexts, such as oocyte meiosis, and may serve as a proxy for investigating the pericentriolar matrix-independent pathway for spindle microtubule generation that supports robust spindle assembly in human cells.

P1001/B2

Deciphering the Cell Cycle's Role in Regulating Centrosome Dynamics - Lessons From Mathematical Modelling.

Z. M. Wilmott, S.-S. Wong, J. Raff;
University of Oxford, Oxford, United Kingdom

Centrosomes form when centrioles recruit an amorphous matrix of pericentriolar material (PCM) around themselves. Studies in flies and worms have revealed that Spd-2/SPD-2, Polo/PLK-1 and Cnn/SPD-5 (fly/worm nomenclature) cooperate to assemble a macromolecular scaffold that expands around the centrioles during mitosis; most, if not all, other mitotic PCM proteins ultimately assemble upon this scaffold. In both systems, Spd-2/SPD-2 appears to recruit Polo/PLK-1, which then phosphorylates Cnn/SPD-5 at multiple sites to stimulate the assembly of a Cnn/SPD-5 scaffold. In flies, centrioles appear to generate a local pulse of Polo activity, which is required to activate Spd-2's ability to recruit Polo, and so initiate mitotic PCM assembly. Here, we derive a spatio-temporal mathematical model to describe how the core embryonic Cdk/Cyclin-dependent cell cycle oscillator coordinates mitotic PCM assembly and disassembly in the early Drosophila embryo. We demonstrate that oscillations in Cdk/Cyclin activity translate into a switch-like behaviour that initiates Cnn-scaffold assembly at moderate levels of activity, maintains the Cnn-scaffold at high levels of activity, and then drives the rapid disassembly of the Cnn-scaffold when Cdk/Cyclin activity drops precipitously at the end of mitosis. We use several experimental approaches to derive estimates for all the key reaction parameters in our mathematical model, and we show that this model captures well the behaviour of the key centrosome scaffolding proteins. By exploring the parameter space of the viscoelastic model, we can predict how the mechanical behaviour of the system varies between a liquid-like and solid-like regime. These studies have the potential to shed important light on the biophysical nature of mitotic centrosomes—in particular whether liquid-liquid phase separation is an important driver of mitotic PCM assembly—a topic that is hotly debated.

P1002/B3

A disease-associated PPP2R3C-MAP3K1 phospho-regulatory module controls centrosome function.

A. Ganga[1], L. K. Sweeney[1], A. R. Ramos[2], C. M. Wrinn[1], C. S. Bishop[1], V. Hamel[2], P. Guichard[2], **D. K. Breslow**[1];

[1]Yale University, New Haven, CT, [2]University of Geneva, Geneva, Switzerland

Centrosomes have critical roles in microtubule organization and cell signaling that underlie their importance in development and disease. However, the mechanisms that regulate centrosome function are not fully defined, and thus how defects in centrosomal regulation contribute to disease is incompletely understood. Using a systems genetics approach, we find here that PPP2R3C, a PP2A phosphatase subunit, is a novel distal centriole protein and functional partner of centriolar proteins CEP350 and FOP. We further show that a key function of PPP2R3C and FOP is to counteract the kinase activity of MAP3K1. In support of this model, *MAP3K1* knockout suppresses growth defects caused by inactivation of *PPP2R3C* or *FOP*, and MAP3K1 and PPP2R3C have opposing effects on basal and microtubule stress-induced JNK signaling. Illustrating the importance of balanced MAP3K1 and PPP2R3C activities, acute overexpression of MAP3K1 severely inhibits centrosome function and triggers rapid disintegration of centrioles. Highlighting the physiologic significance of these findings, inactivating *PPP2R3C* mutations and activating *MAP3K1* mutations both cause congenital syndromes characterized by gonadal dysgenesis. As a syndromic *PPP2R3C* variant is defective in centriolar localization and binding to centriolar protein FOP, we propose that imbalanced activity of this centrosomal kinase-phosphatase pair is the shared cause of these disorders. Lastly, we note that a functional ortholog of the PP2A-PPP2R3C-FOP-CEP350 complex regulates the microtubule cytoskeleton in plants, which lack centrioles entirely. Thus, our findings reveal an evolutionary ancient module that has been conserved across highly divergent modes of microtubule organization. Collectively, our study identifies a new centrosomal phospho-regulatory module, sheds light on disorders of gonadal development, and illustrates the power of systems genetics to reveal previously unrecognized gene functions.

P1003/B4

Microtubule-Based Condensate Transport Contributes To Centrosome Clustering In Cancer Cells.

P.-P. Chen[1,2], A. Saju[1], K.-C. Hsia[1,2];
[1]Academia Sinica, Taipei, Taiwan, [2]National Yang_Ming Chiao Tung University, Taipei, Taiwan

The centrosome, a membrane-less organelle, undergoes amplification in various cancers, contributing to aneuploidy. Cancer cells prevent multipolar spindle formation and subsequent apoptotic cell death by clustering extra centrosomes into a pseudo-bipolar spindle, ensuring accurate cell division. Our study demonstrates that at mitotic entry, HSET, a low-processivity kinesin motor, facilitates transport and coalescence of centrosome-like condensates formed by CDK5RAP2, thereby reducing cytoplasmic puncta. Subsequently, these condensates allocate to form the spindle poles. Total internal reflection fluorescence (TIRF)-based in vitro reconstitution experiments reveal that HSET and CDK5RAP2 co-condensate, enhancing their processivity on microtubules. HSET directly transports CDK5RAP2 condensates towards the microtubules minus ends, converging at interphase microtubule radical arrays and mitotic spindle poles in animal cells. Computer simulations suggest that elevated HSET number and processivity promote centrosome clustering in HSET-over-expressing cancers. Our findings highlight a microtubule-dependent mechanism that regulates the cellular localization of biomolecular condensates.

P1004/B5

Microtubule and centrosome remodeling in quiescence.

A. Massoni-Laporte, D. Laporte, E. Sazli, C. Lefranc, I. Sagot;
CNRS, Bordeaux, France

Cells are constantly facing decision to proliferate or enter in a non-proliferating state. Quiescence is defined as a temporary absence of proliferation, and is the most widespread cellular state on Earth, ranging from prokaryotic to eukaryotic organisms. Quiescence establishment, maintenance and exit, by balancing cell proliferation, are key steps involved not only in normal development and tissues homeostasis, but also in major human pathologies such as cancers. Quiescence is at the heart of the aging process, as over time cells must overcome the deleterious effects of constant increase in damaged macromolecules while maintaining their ability to reproliferate. Recently, it has been shown that upon quiescence establishment, microtubules are reorganized and form a stable structure called Q-nMT bundle that is required for cell survival in quiescence and that controls the return into the proliferative state. We have investigated the relationship between the Q-nMT bundle and the centrosome from which it originates. I have shown that the yeast centrosome is modified upon quiescence establishment, with an increased recruitment of gamma- TuRC components. We are currently searching for possible post-translational modifications of gamma-TuRC and partners that may be involved in Q-nMT bundle formation. Finally, upon exit from quiescence, I have shown that the Q-nMT bundle disassembly is mandatory for centrosome separation upon re-entry into mitosis. We propose that, like primary cilia in metazoans, the Q-nMT bundle may act as a checkpoint to allow quiescence exit, thereby opening new avenues for understanding the interdependence between the primary cilium and quiescence.

P1005/B6

NuSAP Mediates CEP57 Recruitment for Proper Centriole Engagement.

S. Zhang, Y.-C. Liou;
National University of Singapore, Singapore, Singapore

The precise coordination of centrosome function and mitotic spindle formation is essential for accurate cell division and chromosome segregation in vertebrates. While the role of microtubule-associated proteins (MAPs) in spindle formation is well established, their contribution to centrosome integrity remains underexplored. Nucleolar and Spindle-Associated Protein (NuSAP), a RanGTP-regulated MAP, is known for its critical function in spindle stability and chromosome segregation, but its role in maintaining centrosomal integrity has not been fully characterized. In this study, we demonstrate that NuSAP depletion leads to multipolar spindle formation and premature centriole disengagement, uncovering a novel function of NuSAP at the centrosome. Our findings reveal that NuSAP localizes to centrosomes and interacts with CEP57, a key pericentriolar material (PCM) scaffolding protein. Depletion of NuSAP destabilizes centriole tubulin structure, resulting in mis-localization of CEP57, PCM disorganization, and subsequent centriole disengagement. This study highlights the essential role of NuSAP in anchoring the CEP57 complex to centrioles and underscores the previously unrecognized involvement of MAPs in maintaining centrosome integrity, providing new insights into the molecular mechanisms governing cell division.

P1006/B7

Structural Organization of the Centriole Lumen by the Inner Scaffold Network.

C. Sala[1], E. S. Atorino[2], M. Würtz[1], A. Neuner[1], P. Partscht[3], T. Hoffmann[2], S. Eustermann[2], E. Schiebel[1];
[1]Center for Molecular Biology of Heidelberg University (ZMBH), Heidelberg, Germany, [2]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, [3]German Cancer Research Institute, Heidelberg, Germany

Centrosomes are the primary microtubule-organizing centers (MTOCs) in eukaryotes, playing crucial roles in cell proliferation, polarity, and ciliogenesis. Centrosomes consist of the centriole, a nine-fold symmetrical microtubule-based cylinder, and the pericentriolar material (PCM). The function of centrosomes is closely tied to centriole integrity. Therefore, defects in centriolar structures can lead to various centrosome-linked diseases, including cancer, neurodevelopmental disorders, and ciliopathies. The inner scaffold is a ring-like substructure in the centriolar lumen that contributes to centriolar integrity. Based on their localization, centriolar proteins such as POC1B, POC5, FAM161A, and CCDC15 have been identified as potential components of the inner scaffold. In this study, we demonstrate that the two human paralogues, POC1A and POC1B, are essential for the proper localization and function of other inner scaffold proteins. Both POC1 proteins share a similar domain architecture, consisting of an N-terminal WD40 domain and a C-terminal coiled coil. These domains facilitate interactions with specific subsets of proteins. The core of the inner scaffold is formed by a WD40-mediated interaction between POC1A and the POC5-centrin complex, which is an elongated structure consisting of four POC5 molecules. Additionally, POC1A and POC1B form a heterodimer through their coiled coils, cross-linking the inner scaffold and anchoring it to the microtubule wall. Loss of either POC1A or POC1B results in mislocalization of inner scaffold proteins and affects centriolar stability, leading to short and fragmented centrioles, similar to the phenotype observed in *POC5*$^{-/-}$ cells. This ultimately leads to mitotic defects. Our data indicate how the WD40 domain and coiled coil region in the POC1 proteins mediate different

interaction patterns and how the inner scaffold is involved in maintaining centriolar stability, emphasizing the importance of centriole structural integrity for centrosome function.

P1007/B8

Architecture of the centriolar A-C linker.

B. Cai[1], J. Xu[1], L. Luo[2], E. Aarts[1], P. Beltrao[1], T. Ishikawa[2], A. Leitner[1], M. Pilhofer[1], **M. Wieczorek**[1];
[1]ETH Zurich, Zurich, Switzerland, [2]Paul Scherrer Institute, Viligen, Switzerland

Centrioles are massive protein assemblies at the cores of centrosomes and basal bodies. A hallmark of centriolar architecture is the A-C linker, which connects the A- and C-tubules of neighbouring microtubule triplets. Despite its identification several decades ago, we do not understand how the A-C linker is built, nor how it crosslinks microtubules in the centriole. Here, we report high-resolution cryo-electron microscopy reconstructions of the native A-C linker bound to centriolar microtubule triplets in *T. thermophila* basal bodies. We discovered that the A-C linker adopts two distinct conformations: an extended form in the proximal centriolar microtubule triplet region, and a narrow form in the distal portion. These conformations are distinguished by unique A- and C-tubule-binding proteins and an alpha-helical bundle that protrudes from the proximal A-C linker into the cell body. We identified several core A-C linker components, which together repeat every 8 nm along the centriole axis via a fishbone-like alpha-helical lattice. The structure of adjacent A and C-tubules shows that the A-C linker forms a bridge with novel triplet-specific B-C junction proteins, allowing us to build the first molecular model of a centriolar microtubule triplet pair. Our results uncover surprising structural and biochemical heterogeneity in centriole-associated complexes and suggest how the A-C linker could confer mechanical properties to centrioles in motile cells.

P1008/B9

A delta-tubulin/epsilon-tubulin/Ted protein complex is required for centriole architecture.

R. Pudlowski[1], L. Xu[1], L. Milenkovic[2], K. Hemsworth[1], T. Stearns[3], J. Wang[1];
[1]Washington University in St. Louis, St. Louis, MO, [2]Stanford University, Stanford, CA, [3]Rockefeller University, New York, NY

Centrioles have a unique, conserved architecture formed by three linked “triplet” microtubules arranged in nine-fold symmetry. The mechanisms by which these triplet microtubules are formed are not understood, but likely involve the noncanonical tubulins delta-tubulin and epsilon-tubulin. Previously, we found that human cells deficient in delta-tubulin or epsilon-tubulin form abnormal centrioles, characterized by an absence of triplet microtubules, lack of central core protein POC5, and a futile cycle of centriole formation and disintegration. Here, we show that human cells lacking either of the associated proteins TEDC1 and TEDC2 have these same phenotypes. Using ultrastructure expansion microscopy, we identified the roles of these proteins and triplet microtubules in centriole architecture by mapping the locations of centriolar proteins throughout the cell cycle. We find that mutant centrioles have normal architecture during S-phase. By G2-phase, mutant centrioles grow to the same length as control centrioles, but fail to recruit inner scaffold proteins of the central core. Instead, the inner lumen of centrioles is filled with an expanded proximal region, indicating that these proteins, or the triplet microtubules themselves, may be required for recruiting central core proteins and restricting the length of the proximal end. During mitosis, the mutant centrioles elongate further before fragmenting and disintegrating. All four proteins physically interact and TEDC1 and TEDC2 are capable of interacting in the absence of the tubulins. These results support an AlphaFold Multimer structural prediction model

for the tetrameric complex, in which delta-tubulin and epsilon-tubulin are predicted to form a heterodimer. TEDC1 and TEDC2 localize to centrosomes and are mutually dependent on each other and on delta-tubulin and epsilon-tubulin for localization. These results indicate that delta-tubulin, epsilon-tubulin, TEDC1, and TEDC2 function together in promoting robust centriole architecture. This work also lays the groundwork for future dissection of this complex, which will provide a basis for determining the mechanisms that underlie the assembly and interplay between compound microtubules and inner centriole structure.

P1009/B10

Dissecting the dynamics of human centriole assembly by correlative cell cycle-centriole cycle ultrastructural analysis.

D. Kong, D. Luvsanjav, J. Loncarek;
National Cancer Institute, NIH, Frederick, MD

The canonical human centriole is built of nine sets ofmicrotubule triplets, the assembly of which, in proliferating cells, starts inthe early S phase immediately after the initiation of nascent centrioles(procentrioles). Procentriole's longitudinal growth continues throughout itsfirst interphase and completes in its second cell cycle. The availableultrastructural analyses indicate that microtubule triplets are present alreadyin procentriole's first mitosis. The correlation of various centriolarcomponents with centriole length, irrespective of the exact cell cycle stages,has recently been published. However, the precise and systematic dissection ofthe dynamics of procentriole growth incorrelation with the cell cycle stages is still lacking. This gap infundamental knowledge needs to be filled to understand the mechanisms thatsynchronize the centriole assembly with the cell cycle. Such synchronizationoperates throughout the lifetime, ensuring centriole, centrosome, and ciliahomeostasis. Here, we report a study in which we systematically analyzedthe status of procentriole microtubules in precisely defined S and G2 cellcycle stages in several human cell lines. We employed a precise cell cycleproliferation marker and conducted correlative live and electron microscopyanalyses in combination with various super-resolution analyses. We found thatboth longitudinal and lateral incorporation of procentriole microtubules istied to cell cycle transitions and driven by the concerted action of severalkinases. We show that experimentally imbalanced activity of these kinases,which de-couples procentriole growth from cell cycle progression, results inthe loss of centrosome homeostasis. Our meticulous approach unravels thefundamental principles underlying centriole timely assembly in healthyproliferating cells.

P1010/B11

Reconstituting organelle assembly on the surface of synthetic beads.

J. Raff, C. Chan, M. Peng, S. Wong, J. Monteiro;
University of Oxford, Oxford, UNITED KINGDOM.

Almost all human cells are born with a single pair of centrioles, comprising an older mother and a closely-engaged younger daughter. In cycling cells, the centriole pair duplicates precisely once every cell cycle: mother and daughters disengage, the daughter matures into a mother, and the two mothers then each give birth to an engaged daughter that grows from their side during S-phase. In mitosis, the two centriole pairs recruit an amorphous matrix of pericentriolar material (PCM) to form two centrosomes that organise the poles of the mitotic spindle. Centrioles probably comprise >100 different types of protein, and mitotic centrosomes probably >400 different types of protein, yet these structures can

rapidly and precisely assemble during each cell cycle—always forming at the right time and place, and always growing to the right size. How do cells achieve this remarkable feat of bioengineering? Recent studies have shown that centriole and centrosome assembly is driven by a small "core" set of conserved proteins. Here we show that centriole and centrosome assembly can be reconstituted on the surface of synthetic beads that have been coupled to an anti-GFP nanobody and injected into *Drosophila* embryos expressing Sas-4-GFP, a core centriole/centrosome-assembly protein (CPAP in humans). These Sas-4-coated beads recruit mitotic PCM and nucleate MTs in synchrony with the endogenous centrosomes in the embryo. Remarkably, they also generate what appear to be *bona fide* centrioles that disengage from the bead surface, organise PCM and MTs, and then duplicate in synchrony with the endogenous centrioles. Thus, simply concentrating Sas-4 on a bead surface appears sufficient to initiate a robust self-assembly process in the embryo that can rapidly and efficiently build centrioles and centrosomes—in coordination with the cell cycle, but independent of the normal centriole template. These studies provide important insights into how cells can build such complicated protein machines so quickly and so accurately.

P1011/B12

Regulation of PCM in C. elegans sperm - a MTOC halo around sperm DNA?.

J. Magescas, R. K. Y. Ng, J. L. Feldman;
Stanford University, Stanford, CA

During cell division, the centrosome acts as a microtubule organizing center (MTOC), nucleating and localizing microtubules through its pericentriolar material (PCM). Although its ability to act as an MTOC is a conserved feature of the centrosome, the molecular regulation of centrosomal MTOC function can vary depending on cell type. In *C. elegans,* the PCM is composed of three essential proteins, PCMD-1, SPD-2/Cep-192, and SPD-5, the functional homologue of CDK5RAP2/Cnn. We previously found that although these proteins are all essential during the first cell division in *C. elegans,* only SPD-5 is essential for MTOC function across cell types in the developing organism. **To understand how MTOC activity is regulated at the centrosome, we concentrated our efforts on understanding SPD-5 regulation in dividing cells**. One context pertinent to the study of PCM regulation is male meiosis. In *C. elegans,* spermatogenesis concludes with the elimination of the spindle pole in a cellular compartment, the residual body (RB), from which the spermatozoon buds off. This residual body effectively acts as a trashcan for the spermatozoon. However, how this process is made possible and how it relates to the typical stages of PCM disassembly remains a mystery. Using live imaging of endogenously tagged proteins, we observed that during spindle pole disassembly, the PCM fragments into smaller PCM complexes, or "packets." The packets remain attached to microtubules and migrate to the residual body, refocusing into a pole. Interestingly, these packets are not further disassembled in the residual body and remain stable through time. Surprisingly, we observed that soon after the spindle pole is eliminated from the spermatozoon, tubulin and SPD-5 progressively surround the sperm DNA, forming a "halo" around the sperm DNA. Interestingly, SPD-2 and PCMD-1 were only observed at the sperm centrioles and in the residual body, but not in the halo. We analyzed different proteins linked to MTOC activity using both live imaging and expansion microscopy and observed that a subset consistently localized similarly, surrounding the DNA. Using a combination of expansion microscopy and cryotomography, we are currently assessing the organization of this halo. Based on preliminary genetic data disrupting these halo proteins, we hypothesize that these sperm-provided proteins might contribute to controlling the timely activation of the centrosome into a spindle pole in the zygote.

P1012/B13

The *C. elegans*WASH complex supports transport and microtubule function.

P. Irizarry[1], B. Ramamurthy[2], M. Soto[1];
[1]RUTGERS University - RWJMS, Piscataway, NJ, [2]School of Health Professions, Rutgers University, Piscataway, NJ

In *C. elegans*, only two Nucleation Promoting Factors (NPSs) that regulate branched actin through Arp2/3 have been examined, WAVE and WASP. We analyzed the orthologs of the third *C. elegans* Apr2/3 NPF, the WASH complex. The WASH complex is similar to the WAVE complex, in that both complexes include 5 paralogous components. Surprisingly, only 4 of the 5 components had been identified in *C. elegans*, and the role of WASH had not been previously described. We used existing mutations, RNAi and CRISPR to determine the effects of loss of the WASH complex, focusing on two epithelial tissues: the adult intestine and the embryonic epidermis. We used two phenotypic assays to test the role of WASH components, including a candidate for the missing 5th WASH component, the FAM21 proposed ortholog, CO5G5.2. (1) Using assays for protein transport showed that loss of any WASH component, using mutations or RNAi, resulted in the same phenotype: defective transport of cargo on RAB-5 and PI(3)P-positive early endosomes. This result suggests *C. elegans* WASH regulates sorting during endocytic recycling, through retrograde trafficking to the Golgi apparatus. (2) Using assays for embryonic development showed that loss of WASH components resulted in embryos that die during morphogenesis, and some that died earlier. Therefore, in contrast to loss of WASP or WAVE, which are needed during morphogenesis, loss of WASH resulted in some embryos that died before morphogenesis begins, due to cell cycle arrests. Beta-tubulin::GFP expression was strongly diminished in these embryos. It is known that the WASH complex assembles at Centrosomes, so that Centrosomes support WASH. We are therefore investigating if WASH feeds back on Centrosome function, to address the effect of WASH on microtubules (MTs). Thus, *C. elegans* has a conserved, 5-member WASH complex that appears to function, like its orthologs, in early endosome to Golgi retrograde transport. In addition, *C. elegans* provides a great system to investigate how the WASH complex coordinates MT-dependent events during early embryonic development.

Actin Cytoskeleton 1

P1013/B14

Unraveling the regulatory mechanism of FNBP4-mediated FMN1 regulation in actin cytoskeleton remodeling.

S. Das, S. Maiti;
Indian Institute Of Science Education & Research Kolkata, Nadia, India

Actin cytoskeleton is crucial for various cellular processes, such as cell motility, endocytosis, and DNA damage repair. These processes require extensive actin remodeling, which is regulated by numerous actin-binding proteins that control the assembly and disassembly of actin filaments. Formins, a bona fide group of actin cytoskeleton regulators, play a pivotal role in filament nucleation and elongation. In mammals, there are 15 formins, broadly classified based on their regulatory mechanisms into diaphanous-related formins (DRFs) and non-diaphanous-related formins. DRFs, such as mDia1 and Daam1, are regulated by Rho GTPase, releasing them from an auto-inhibited conformation. In contrast,

Formin1 (FMN1), a key member of the non-diaphanous formins, is not regulated by Rho GTPase, and the regulatory mechanisms for non-diaphanous formins remain largely uncharacterized. Therefore, we aimed to investigate the regulation of non-diaphanous formin FMN1. In our previous study, we identified an interaction between the N-terminal WW1-WW2 domains of FNBP4 (Formin-binding protein 4) and the C-terminal FH1-FH2 domains of FMN1. Surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA) demonstrated that the WW1 domain of FNBP4 specifically interacts with the FH1 domain of FMN1. Deletion of the WW1 domain (resulting in the N-ter ΔWW1 FNBP4 construct) abolished this interaction. Here, we aim to unravel the functional implications of the FMN1-FNBP4 interaction in actin cytoskeleton dynamics regulation. Using total internal reflection fluorescence (TIRF) microscopy and pyrene-actin polymerization assays, we found that FNBP4 inhibits FMN1-driven actin assembly in vitro. Furthermore, in the actin filament elongation assay, FNBP4 inhibits processive activity of FMN1, preventing it from displacing the capping protein CapZ at the growing barbed ends of actin filaments. We also found that the WW1-WW2 FNBP4 inhibits the bundling activity of the FH1-FH2 FMN1 in a concentration-dependent manner, while not affecting the bundling activity driven by the FH2 FMN1. In summary, we propose that FNBP4 functions as a stationary inhibitor of FMN1. This study can lead to the discovery of regulatory mechanisms specific to non-diaphanous formins, contributing to a deeper understanding of actin dynamics and providing new mechanistic insights.

P1014/B15

Rap1 and Rap2 GTPases Regulation and Function Through ARHGAP29 to Establish Cell Polarity and Promote Cell Migration.

A. Neumann, R. Prekeris;
University of Colorado Anschutz Medical Campus, Aurora, CO

Cell migration is a dynamic and highly regulated process involving polarization of the migrating cell into a lagging and leading edge. These distinct edges are mainly established through the formation of branched actin networks at the leading edge and actin-myosin stress fibers at the lagging edge. We have focused on two members of the Ras family of GTPases, Rap1 and Rap2, as important regulators of cell polarity and the actin cytoskeleton. Both Raps display unique mechanisms of activation and localization regulation. Rap1 and Rap2 bind to a Rab40 E3 ubiquitin ligase complex which causes them to be tri-mono-ubiquitinated and activated. Ubiquitinated Rap2 is localized to the plasma membrane while non-ubiquitinated Rap2 is localized to lysosomes. Rap1 ubiquitination results in Rap1 localization to the cytoplasm while loss of Rap1 ubiquitination drives Rap1 localization away from the cytoplasm and to the plasma membrane. Interestingly, co-overexpression of both Rap1 and Rap2 results in Rap2 localization to lysosomes indicating that there is antagonism between Rap1 and Rap2 localization. To study the role of the Rap GTPases in actin regulation and cell migration, we made cell lines with either Rap1b loss or Rap2 loss. Interestingly, loss of Rap1b hyperpolarizes cells and impairs chemotactic migration. Loss of Rap2 disrupts cell polarity and prevents cells from migrating without a chemotactic stimulus. Further work in understanding how Raps are influencing the actin cytoskeleton and cell migration has suggested that they oppositely regulate lamellipodia localization of ARHGAP29, a putative RhoA GAP previously linked to regulating RhoA activity in cell-cell adhesions. ARHGAP29 has been linked to metastasis of multiple cancer types, however, how it functions in cell migration remains unclear. We have found that loss of ARHGAP29 abolishes cell polarity, resulting in cells with severely hindered random and chemotactic migration capabilities. Interestingly, these ARHGAP29 KO cells do not display major defects in dynamic ruffling actin, indicating that ARHGAP29 plays a role in localization of actin ruffling but not actin ruffling dynamics. These data suggest a mechanism by which Rap1 and Rap2 antagonistically

localize ARHGAP29 to the plasma membrane, allowing for ARHGAP29 activity as a RhoGAP to promote ruffling actin and thus polarize cells to form a lamellipodia.

P1015/B16

Cell-cell signaling makes an essential contribution to apical constriction by basolaterally recruiting Arp2/3 via Rac and WAVE.

P. Zhang, T. Medwig-Kinney, E. Breiner, J. Perez, A. Song, B. Goldstein;
UNC At Chapel Hill, Chapel Hill, NC

Apical constriction is a critical cell shape change that sculpts tissues into specific forms during development. How precisely-localized actomyosin regulators drive apical constriction remains poorly understood. *C. elegans* gastrulation provides a genetically and optically amenable model to address this question. The Arp2/3 complex has been shown to play essential roles during *C. elegans* gastrulation. To understand how Arp2/3 is locally regulated, we imaged embryos with endogenously-tagged Arp2/3 and nucleation-promoting factors (NPFs). The three *C. elegans* NPFs - WAVE, WASP, and WASH - showed distinct subcellular locations. Further, the NPFs colocalized with Arp2/3 and controlled Arp2/3 localization at each of these subcellular locations. These results indicate that three NPFs modulate Arp2/3 at different subcellular locations, which we have exploited to study the contributions of distinct populations of Arp2/3 in apical constriction. We found that WAVE depletion leads to highly penetrant gastrulation defects, WASP makes a fully redundant contribution, and WASH depletion did not cause noticeable defects. WAVE localizes basolaterally at the site of cell-cell contacts, and this localization is dependent on Rac (CED-10). We further found that establishing ectopic cell-cell contact between embryos is sufficient to recruit WAVE and Arp2/3, indicating that cells use cell-cell contacts as a symmetry-breaking cue for the localization of these proteins. We also noticed that CED-10, WAVE, and Arp2/3 depletion caused membrane blebs in early embryos. Because bleb formation depends on myosin activity, we asked if the gastrulation defects in WAVE-depleted embryos could be rescued by tuning myosin levels. We found that reducing myosin levels to 30-50% could restore normal membrane morphology and rescue the gastrulation defects in 40-70% of the embryos. Taken together, our results suggest that Rac signaling basolaterally, at cell-cell contacts, is essential for maintaining proper membrane-cortex linkage and contributes to the success of apical constriction.

P1016/B17

Actin-binding Protein as a Novel Damage-Associated Molecular Pattern.

J. Antonello, D. Gau, I. Eder, A. VanDemark, P. Roy;
University of Pittsburgh, Pittsburgh, PA

Damage-associated molecular patterns (DAMPs) are molecules known to be released from damaged or dying cells in response to stress. These molecules then activate the innate immune system, often by interacting with toll-like receptors (TLRs) present on immune cells. Although actin-binding protein profilin1 (Pfn1) is best known for its intracellular role as a regulator of actin cytoskeletal dynamics, we previously demonstrated that a small fraction of cellular Pfn1 is also externalized by various cells. However, what signals drive Pfn1 externalization and the biological significance of externalized Pfn1 remain unclear. In this study, we show that induction of genotoxic and endoplasmic reticulum (ER) stresses increases cellular Pfn1 mRNA expression and facilitates Pfn1 release in the extracellular milieu. We further show evidence for soluble Pfn1's binding to the cell membrane and induction of phenotypic changes when added in the extracellular space. Importantly, extracellular Pfn1 stimulates the expression

of pro-inflammatory cytokines/chemokines (IL1β, IL-6, and CXCL10) in macrophages in a dose-dependent manner and this effect is diminished significantly upon pharmacological inhibition of Myd88, a critical cofactor for TLR signaling. Collectively, these findings raise the possibility that cytoskeleton-regulatory proteins may be a novel class of DAMPs with downstream immunomodulatory action. This study could have a broad implication of extracellular Pfn1's role in the context of chronic inflammation for multiple disease types to be investigated in the future.

P1017/B18

TDP-43 Regulates Actin Dynamics.

A. D. Nobles, L. M. Haney, B. K. Haarer, J. L. Henty-Ridilla;
SUNY Upstate Medical University, Syracuse, NY

Amyotrophic lateral sclerosis (ALS) is an untreatable neurodegenerative disease where patients experience a persistent decline in motor functions. Trans Active Response (TAR) DNA-binding protein 43 (TDP-43) is implicated in almost all cases of ALS and to a lesser extent in several other neurodegenerative diseases. TDP-43 a biomolecular condensate-forming, multifunctional protein that performs diverse motor neuron cell functions including RNA metabolism, mitochondrial processes, and stress response which become disrupted in patients with ALS. While the actin cytoskeleton or its dynamics has not been directly connected to motor neuron dysfunction in ALS, it shares several essential cellular functions with TDP-43. Here we use biochemical assays with purified proteins to characterize a previously unknown direct interaction between TDP-43 and actin. We use total internal reflection fluorescence (TIRF) microscopy to visualize TDP-43 directly interacting with and altering in vitro actin polymerization, significantly reducing actin nucleation and slowing filament elongation. Therefore, TDP-43 partially inhibits actin polymerization.

P1018/B19

VopF is a processive polymerase of barbed and pointed ends of actin filaments.

A. Arya[1], E. Kudryashova[2], D. Kudryashov[2], S. Shekhar[1];
[1]Emory University, Atlanta, GA, [2]The Ohio State University, Columbus, OH

Actin is an essential protein required for force generation in key cellular processes including cytokinesis, cell migration, phagocytosis etc. Intracellular actin networks are thought to assemble by polymerization of filaments at their barbed ends and depolymerization at their pointed ends. This process, referred to as "treadmilling", forms the central bedrock of our current understanding of actin dynamics. Recent results from our lab challenge this fundamental paradigm. Using a combination of multicolor single-molecule and single filament experiments we have recently discovered the first ever processive polymerase that can elongate barbed as well as pointed ends. To elucidate the underlying mechanism of VopF-mediated polymerization, we propose a two state model where VopF can exist in a polymerization competent and a polymerization incompetent state. To test this model, we applied flow-induced pulling forces on filaments elongating from surface-anchored VopF molecules. Our results show that VopF's polymerization function is mechanosensitive i.e., its rate of polymerization increases under pN-range pulling forces which supports our two state model. Our new findings challenge the basic tenets of our current understanding of intracellular actin dynamics and call for reevaluation of previously established paradigms of actin assembly.

P1019/B20

Liquid-like Condensates that Bind Actin Drive Filament Polymerization and Bundling.

C. Walker[1], A. Chandrasekaran[2], D. Mansour[2], K. Graham[1], L. Wang[3], E. M. Lafer[3], P. Rangamani[2], J. C. Stachowiak[1];
[1]University of Texas At Austin, Austin, TX, [2]University of California San Diego, La Jolla, CA, [3]University of Texas Health Science Center at San Antonio, San Antonio, TX

The actin cytoskeleton forms filament networks that play a critical role in cell motility, endocytosis, and adhesion. The assembly of this network is facilitated by actin accessory proteins, which collectively determine filament network characteristics. Several cytoskeletal proteins have been shown to assemble into condensates via liquid-liquid phase separation, a process through which biomolecules self-assemble into a liquid-like condensed phase. Building on these findings, we recently showed that condensates consisting of the actin polymerase VASP can polymerize and bundle actin filaments. As actin polymerized inside VASP condensates, actin filaments accumulated at the inner surfaces of the condensates to minimize filament curvature. This partitioning led to the assembly of a peripheral, ring-like bundle of actin within condensates. When the rigidity of the actin ring overcame the surface tension of the VASP condensate, the filaments spontaneously straightened, deforming the initially spherical VASP condensates into rod-like structures filled with parallel bundles of actin filaments. Building on these findings, our ongoing work illustrates that actin polymerization and bundling by protein condensates do not require proteins with specific polymerase activity. Specifically, we show that condensates composed of Lamellipodin, a dimeric protein that binds actin but is not an actin polymerase, could also polymerize and bundle actin filaments. Additionally, we found that a monomeric form of Lamellipodin was also capable of forming condensates that polymerized and bundled actin. How does the formation of protein condensates confer this capacity upon Lamellipodin? Multi-valent binding to actin filaments is thought to underlie the function of canonical actin polymerases. Therefore, one possible explanation is that the condensate environment promotes multivalent interactions between Lamellipodin and actin. To investigate the potential contribution of protein condensates to actin bundling, we developed an agent-based model of filament rearrangement within spherical containers that mimic protein condensates. Guided by its predictions, we hypothesized that any condensate-forming protein that binds actin could bundle filaments. To test this idea, we added an actin-binding motif to Eps15, a condensate-forming protein that does not normally bind actin. The resulting chimera formed condensates that drove efficient actin polymerization and bundling. Collectively, these results demonstrate that filament polymerization and bundling are emergent properties of liquid-like protein condensates that bind actin, suggesting a general principle of actin organization through multivalent interactions.

P1020/B21

Capping Protein Mediates Competition among F-Actin Networks and Competes with Formin to Tune Network Architecture and Dynamics in the *C. elegans* Zygote.

S. E. Yde, R. S. Kadzik, C. Suarez, E. Munro[,1,1], D. R. Kovar[,1,1,1];
University of Chicago, Chicago, IL

Eukaryotic cells assemble diverse filamentous (F-) actin-based networks, with distinct architectures and dynamics, to perform numerous essential cellular tasks. The properties of these highly specialized networks are specified by the activities of different actin binding proteins (ABPs) including actin assembly factors such as formin and the arp2/3 complex, bundlers, and regulators of actin filament

length, including Capping Protein (CP). Previous studies have established that competition among networks for a limited pool of actin monomers and ABPs helps coordinate the assembly of different F-actin networks. Here, we show that CP regulates this competition in the one cell *C. elegans* embryo (zygote). Additionally, within a network, CP competes with formin to limit actin filament elongation, thus regulating the initiation and maintenance of assembly of that network. Using *in vivo* multicolor near-TIRF microscopy, we simultaneously visualized formin, arp2/3 complex, and CP to characterize two F-actin networks in the zygote: filopodia and mini-comets. Filopodia are membrane-bound protrusions containing a shaft of bundled filaments assembled by formin and a base assembled by the arp2/3 complex, while mini-comets are assembled by the arp2/3 complex. We show that CP is dynamically enriched at the base of filopodia and throughout mini-comets. Depleting CP via RNAi increases the frequency and duration of filopodia assembly, while decreasing mini-comet assembly, suggesting that CP regulates the competition for resources between these two networks. The increase in filopodia assembly upon CP RNAi is reflected in an increase in initiation of filopodia and two-fold increase in filopodia lifetime.To understand the mechanisms that drive these changes, we used high-speed imaging to quantify the movements of formins along the shafts of filopodia in control and CP-depleted zygotes. We observe rapid movements of formins toward filopodia tips and slower movements away from the tips, suggesting that filopodia are maintained by a balance of initiation and termination of actin filament assembly in the filopodia shaft. In CP-depleted zygotes, the frequency of formin initiation is increased, suggesting that CP regulates initiation rates via competition with formin for binding barbed ends in the filopodia base, thus influencing filopodia lifetime. Together, these results reveal a role for CP in regulating the coordination between arp2/3 complex and formin to assemble and maintain dynamic F-actin based structures in the context of a crowded cytoplasm.

P1021/B22

TRIM9 Regulates Melanoma Adhesion, Actin Dynamics, and Motility.

K. Lukasik, J. Brooks, J. Bear, S. Gupton;
UNC Chapel Hill, Chapel Hill, NC

Cell shape change and motility involve remodeling of the actin cytoskeleton, cell adhesions, and plasma membrane. How these cytoskeletal and membrane remodeling are altered in pathological states remains largely unknown. Netrin is an extracellular morphogen which promotes neuronal morphogenesis and cancer progression. Here we examine the role of a brain enriched E3 ubiquitin ligase TRIM9 implicated in netrin dependent neuronal morphogenesis in the context of melanoma. We previously identified that TRIM9 regulates netrin dependent actin dynamics and exocytosis in developing neurons. Deletion of murine *Trim9* impairs neuronal migration, netrin induced axon turning, and axonal and dendritic branching, and increases exocytosis and filopodial stability. TRIM9 alters the dynamics of the actin polymerase VASP at filopodia tips via non degrative ubiquitination. TRIM9 is expressed in other motile cells, but the non neuronal role of TRIM9 is unknown. TRIM9 was identified as a potential prognostic biomarker in melanoma and high TRIM9 expression correlates with low patient survival. Melanomas undergo phenotype switching, where three distinct phenotypes exist that are associated with differential gene expression. Single cell RNAseq data from patient-derived melanoma indicate TRIM9 is highly expressed in phenotypes that correlate with poor prognosis. *Broadly our findings support the hypothesis that TRIM9 coordinates actin dynamics, adhesion, and exocytosis in melanoma to regulate cell motility and potentially invasion.* We show that in several human melanoma lines TRIM9 protein is enriched and netrin is secreted. Here we examine the role of TRIM9 in regulation of focal adhesions, exocytosis, migration, and invasion. Genetic loss of *TRIM9* increased random

migration velocity, but reduced directional persistence. We find that TRIM9 plays a role in promoting bleb like morphology and inhibits the ability of cells to durotax on soft shallow. Fluorescence recovery after photobleaching, Total internal reflection fluorescence and widefield microscopy revealed that loss of TRIM9 results in increased focal adhesions, cell size, and altered dynamics of focal adhesion proteins VASP, zyxin, and paxillin dynamics. TRIM9 knockout cells exhibit reduced filopodial length and density and altered filopodial localization of VASP and lamellipodial width. Zymography indicates that TRIM9 knockout cells display an increased degradative capacity. Current studies investigate how loss of TRIM9 alters parameters of cell contractility, exocytosis and *in vivo* metastasis to define the role of TRIM9 in melanoma motility. These findings suggest TRIM9 reduces adhesion and migration, increases proliferation and blebbing, and may be an important regulator of phenotype switching in melanoma.

P1022/B23

Isoform Specificity of a Compound Targeting Actin Filaments Containing Tropomyosin Tpms1.8 and 1.9.

J. Hook, E. C. Hardeman, **P. W. Gunning**;
University New South Wales, Sydney, Australia

The unbranched actin filaments in mammalian cells are usually composed of co-polymers of a specific tropomyosin with actin. Microscopy and biochemistry techniques have demonstrated that the different tropomyosin isoforms are spatially segregated in a wide range of cells. Furthermore, genetic manipulation has revealed that the tropomyosins largely define the functional properties of actin filaments in an isoform-specific non-redundant manner. Hence, the development of compounds that target different tropomyosins are potentially valuable tools for cell biology as well as potential therapeutics. New generation compounds that target Tpm1.8/1.9, Tpm3.1/3.2 and Tpm4.2 have been developed and published. These compounds are all based on targeting differences in the N-terminus of these tropomyosins encoded by exon 1b. We have addressed the isoform specificity of the compound that targets the isoforms Tpm1.8 and Tpm1.9. These isoforms are identical except for an internal alternatively spliced exon. Tpm1.8/1.9 is primarily enriched in the lamellipodium of migrating cells but not in stress fibre bundles unlike Tpm3.1/3.2 and Tpm4.2 that are enriched in stress fibres. Exposure of fibroblasts and SK-N-SH cells to the compound Tpm189-3 (PubChem CID 18973468) results in dispersal of Tpm1.8/1.9 from the lamellipodium to a diffuse organisation in the cytoplasm. In contrast, at doses that disperse Tpm1.8/1.9, the compound has no impact on the association of either Tpm3.1/3.2 or Tpm4.2 with actin filament bundles. We also tested the impact of Tpm189-3 on cancer cell viability since compounds that target Tpm3.1/3.2 are highly cytotoxic to cancer cells. Tpm189-3 does not impact cancer cell viability at doses over 5-fold greater than the dose required to disperse the lamellipodial organisation of Tpm1.8/1.9. In addition, exposure of fibroblasts to Tpm189-3 at doses that disperse Tpm1.8/1.9 impact the polarity of these cells and lead to elongation with reduce width. Live imaging identifies a loss of persistent directional movement potentially as a result of disrupted cell polarity. We conclude that the amino acid sequence differences located at 7 positions in the first 16 residues of these isoforms provides sufficient specificity to generate compounds that target Tpm1.8/1.9 alone.

P1023/B24

Turnover Mechanisms of Nonmuscle Myosin IIA and IIB in Cells.

A. Chougule, T. Svitkina;
University of Pennsylvania, Philadelphia, PA

Nonmuscle myosin II (NMII), essential for cellular contractility, comprises three isoforms—NMIIA, NMIIB, and NMIIC—which homo- and heteropolymerize to form NMII bipolar filaments. The specific interplay between these isoforms and their contributions to actin cytoskeletal organization remain unclear. We previously demonstrated that ectopic NMIIA expression in COS-7 cells, which lack NMIIA, redistributes NMIIB-dominated stress fibers. We hypothesized that NMIIA that is characterized by faster turnover of bipolar filaments, can dynamize the NMIIB filaments through copolymerization. However, the specific determinants of the NMIIA molecule responsible for its fast turnover are not fully defined. Here, we systematically analyzed the ability of NMIIA and NMIIB mutants to affect the NMIIB dynamics and distribution in COS-7 cells.
Using overexpression of the various forms of NMIIA and NMIIB that contained mutations expected to impair or enhance the turnover of these myosins, we showed that the NMIIA tail domain is a key driver of endogenous NMIIB redistribution and that both the presence of the NMIIA nonhelical tail (NHT) and phosphorylation of S1915 and/or S1916 are required to preserve the NMIIA's ability redistribute the endogenous NMIIB in COS-7 cells, whereas individual mutations (ΔNHT or S1915/1916/1943A) minimally impair the NMIIB distribution. In agreement, FRAP analysis revealed that the NMII versions capable of redistributing NMIIB exhibited faster recovery rates as compared with the constructs that failed to redistribute NMIIB. This underscores the importance of both the NHT and the C-terminal phosphorylation sites of NMIIA in regulating NMIIB distribution and suggests that a combination of these mechanisms allows NMIIA to exhibit fast filament turnover. Conversely, the "dynamizing" S1935D mutation in the NMIIB tail allows this mutant to redistribute endogenous wild type NMIIB, thus mimicking the effect of exogenous NMIIA. Collectively, our findings provide insights into how isoform-specific regulation of NMII filament turnover affects the organization and dynamics of the cellular contractile system.

P1024/B25

Actin filament assembly driven by surface-associated polymerases: a quantitative theory.

D. Mullins, K. Skruber;
University of California, San Francisco, San Francisco, CA

Actin filaments created by the Arp2/3 complex form branched networks, whose growth can move and shape cellular membranes. Here, we show how membrane surfaces can locally accelerate filament assembly via clustering of proteins that bind actin monomers and/or profilin-actin complexes. Although effects on monomer orientation or 'steering' may enhance the rate of filament elongation, polymerase activity can be explained solely by clustering of polymerizable actin. Briefly, thermal fluctuations of the filament tip define a region of interaction with the membrane. Delivery of actin from solution to this region follows the formalism of Berg (1970), while delivery of actin to the growing filament is driven by two-dimensional diffusion of the filament tip across the membrane. We calculate the dependence of polymerase activity on both the surface density of actin-binding proteins and the solution concentration of polymerizable actin, and show that surface-mediated polymerization outpaces solution-mediated elongation, even at high concentrations of soluble actin (>100 μM). Finally, we expand our formalism to

describe the effects of barbed-end crowding, profilin dissociation, and compressive forces on surface mediated polymerization.

P1025/B26

A Role for WAVE Regulatory Complex in Bone Homeostasis and Osteoclast Function.

S. Das[1], S. Stahnke[1], E. Werbenko[2], D. JJ de Gorter[2], B. Dankbar[2], T. Stradal[1];
[1]Helmholtz Centre for Infection Research, Braunschweig, Germany, [2]Institute of Musculoskeletal Medicine, University Hospital Muenster, Muenster, Germany

Hem1 is the hematopoietic specific paralog of Nap1 and embodies an essential subunit of the WAVE regulatory complex (WRC), necessary for Rac1-mediated lamellipodial protrusion. We generated Hem1 knockout ($Hem1^{-/-}$) mice that develop an autoimmune disease (Salzer *et al.,* 2020) and concurrently display abnormal bone phenotypes reminiscent of osteopetrosis. Bone homeostasis is maintained by a balance between osteoblast and osteoclast activity. Osteoblasts descend from the mesenchymal lineage and express the paralogous Nap1, while osteoclasts originate from the hematopoietic monocytic/macrophage lineage, exclusively expressing Hem1. Our flow cytometry data reveal that $Hem1^{-/-}$ macrophages have a higher tendency to differentiate into osteoclasts compared to wild-type macrophages upon RANKL (Receptor Activator of Nuclear factor Kappa-β Ligand) stimulation. This is further corroborated by data obtained from live cell imaging uncovering that $Hem1^{-/-}$ macrophages differentiate faster into osteoclasts than their wild-type counterparts. *In vitro* studies also show that cell size and consequently the number of nuclei in osteoclasts differentiated from $Hem1^{-/-}$ bone marrow is significantly increased, indicating pronounced cell fusion. We additionally studied the expression of osteoclast-specific marker proteins such as RANK (receptor activator of nuclear factor-κB), or DC-STAMP (dendritic cell-specific transmembrane protein) in $Hem1^{-/-}$ and wild-type osteoclasts, uncovering an altered expression pattern at different stages of osteoclast differentiation, which is in line with altered differentiation. Moreover, $Hem1^{-/-}$ osteoclasts show a reduced ability to degrade bone matrix and immunofluorescence studies elucidate that this is accompanied by impaired ruffled border and discontinuous actin belt formation. We have shown that WRC deficiency causes an integrin activation defect in $Hem1^{-/-}$ macrophages (Stahnke *et al.,* 2021). To explore whether this also applies to osteoclasts and/or contributes to the phenotype, we probed the effects of selected extracellular matrices on osteoclast differentiation and found indeed significant differences. This study offers a new perspective on the role of Hem1 and in particular WRC in general on cell differentiation. In addition, these insights might aid the development of strategies to tackle bone related disease like osteopetrosis.

P1026/B27

A Sonic Hedgehog Deployment Complex Signals to Induce Cytoneme Formation.

C. Daly, E. Hall, C. Hom, S. K. Ogden;
St Jude Children's Research Hospital, Memphis, TN

Embryonic development relies on intercellular communication via secreted morphogens. These signaling ligands facilitate pathway activation over long distances to instruct cell fate and pattern tissues. Sonic Hedgehog (SHH) morphogens play integral roles in a variety of developmental processes including neural tube patterning. SHH-producing cells extend long, actin-based “cytonemes” that facilitate direct transport of ligand to signal-receiving cells. However, these delicate signaling filopodia do not survive conventional fixation methods. To permit mechanistic studies on cytonemes in mammalian systems, our lab developed protocols preserving cytonemes for confocal analysis in cultured cells. Using these

techniques, we found that expression of SHH promotes cytoneme formation in Mouse Embryonic Fibroblasts (MEFs). We generated compound receptor knockout MEFs to determine the receptor requirements for SHH-stimulated cytoneme outgrowth. Based on these results, we describe here a novel SHH Deployment Complex (SDC), consisting ofSHH binding to DISP deployment receptor and at least one of the SHH coreceptors CDON or BOC. SHH binding to the SDC stimulates actin polymerization forcytoneme outgrowth. Since knockout of both BOC and CDON ablates SHH-induced cytonemes in MEFs, one of the primary functions of these coreceptors within theSDC is activation of cytoneme-initiating signaling downstream of SHH-coreceptor engagement. Using RNAscope *in situ* analysis at progressive embryonic time points, we found that CDON, not BOC, is expressed in the SHH-producing tissue of the developing mouse neural tube. Therefore, we conclude that DISP and CDON comprise the SDC in neural tube development. To better understand the mechanistic function of the SDC in cytoneme initiation signaling, we mapped the interaction between DISP and CDON and found that these receptors can interact through their intracellular domains. Binding of SHH to DISP causes conformational shifts in the C-terminal tail of DISP, alleviating this interaction and rendering CDON accessible for recruitment of actin-polymerization machinery. To assess the requirement for CDON in the generation of SHH-transporting cytonemes *in vivo*, we developed a protocol to preserve cellular extensions in the developing mouse neural tube for confocal analysis.

P1027/B28

Tensins are Regulated by Clusterin to Modulate Actin Dynamics and Extracellular Matrix.

P. P. Pattabiraman, A. Shivashankar, A. Soundararajan;
Indiana University School of Medicine, Indianapolis, IN

In our previous study, we demonstrated that the knockdown of clusterin, a secretory chaperone protein, enhanced actin polymerization with increasing cell adhesive interactions and extracellular matrix (ECM) levels and constitutive induction of clusterin expression decreased actin polymerization in trabecular meshwork (TM) cells. The TM tissue, found in the anterior segment of the eye, is composed of smooth muscle type of biomechanically sensitive cells, which aids in sensing and regulating the intraocular pressure. Actin cytoskeleton tension, cell adhesive interactions, and ECM stiffness play important roles in the pressure-sensing function of the TM. Therefore, this study aimed to elucidate the mechanistic evidence for clusterin gain-of-function on the actin and cell adhesive interactions in human TM (HTM) cells. To study this, we performed - A) tandem mass spectrometry-based global proteomic analysis on HTM cells constitutively expressing clusterin (AdCLU) compared to control (AdMT). Screening criteria protein level changes included $p \leq 0.05$ and mean$\pm 2\sigma$ of $\log2 \geq 0.3$ as the confidence fold change (FC). B) Label-free proteomic analysis of co-immunoprecipitated (Co-IP) samples with tensin 3 (TNS3) antibody to determine its interactors. C) The effect of siRNA-mediated loss of TNS3 (siTNS3), and the effect of pcDNA-EGFP-TNS3 (eTNS3)-mediated overexpression on actin fibers. D) Immunoblotting (IB) to analyze the effect of the siTNS3 on actin-associated proteins. Statistical analyses were performed using a student's t-test with significance at $p \leq 0.05$. Proteomic analysis showed AdCLU significantly reduced levels of actin alpha 1 ($p=0.046$, log2FC=-0.5), TNS1 ($p= 0.08$, log2FC=-0.23), TNS3 ($p=0.02$, log2FC=-0.44), and TNS4 ($p=0.04$, log2FC=-0.22) suggesting clusterin-mediated control of cytoskeleton and cell adhesive interactions. Confirmatory analysis by IB showed that AdCLU significantly reduced TNS3 ($p=0.01$). Proteomics of Co-IP samples using TNS3 antibody revealed interactions of TNS3 with major cytoskeletal and ECM proteins, including vimentin, actin, filamin-A, twinfilin-2, and tenascin-c. To better understand the functional role of TNS3, knocking down TNS3 using siTNS3 reduced F-actin distribution compared to siScramble (siScr). Further, IB analysis in siTNS3 compared to siScr showed a significant

downregulation of fibronectin (p=0.04) and vimentin (p=0.03). Contrarily, induction of TNS3 using TNS3 expression plasmid demonstrated increased F-actin and increased distribution of active integrin β1 and β3 compared to control. This novel study is a firsthand report on how clusterin regulates actin and the integrin-adhesome complex regulators like the tensins, which can modify fibrillogenesis via integrins and alter tissue biomechanics.

P1028/B29

Overexpression of Lifeact in the *C. elegans* body wall muscle causes sarcomere disorganization and embryonic or larval lethality.

S. Ono;
Emory University, Atlanta, GA

Live imaging of the actin cytoskeleton has been technically advanced by development of probes for actin filaments. Lifeact, a short peptide derived from yeast ABP140, is one of the most widely used F-actin probes. Although Lifeact is useful to label many actin-based structures in cells, several studies have shown that overexpression of Lifeact can be detrimental to normal biological processes in which actin plays a major role. Biochemical and structural studies have shown that Lifeact can slow down actin dynamics and compete with other proteins for F-actin binding. Here, I report effects of Lifeact on sarcomeric actin organization in the nematode *Caenorhabditis elegans* body wall muscle, which is an obliquely striated muscle with distinct sarcomeres. When mCherry::Lifeact was specifically expressed in the body wall muscle at low levels, it labeled sarcomeric actin filaments without noticeable abnormalities. However, when mCherry::Lifeact was overexpressed, actin filaments were severely disorganized into abnormal bundles in the muscle cells. Remarkably, worms carrying extrachromosomal arrays containing the transgene for muscle-specific expression of mCherry::Lifeact were ~40 % lethal either at late embryonic stages (~30 %) or the L1 larval stage (~10 %). Therefore, severe disturbance of the actin organization in the body wall muscle can cause developmental arrest of the whole animal. In contrast, RHS41, a strain carrying a single-copy transgene for muscle-specific expression of Lifeact::mRuby (Higuchi-Sanabria et al., 2018), was rarely lethal with a low-expression level of Lifeact::mRuby properly labeling sarcomeric actin filaments, indicating that this strain is suitable for live imaging of actin filaments in *C. elegans* muscle. In conclusion, overexpression of Lifeact in *C. elegans* can cause severely detrimental effects on actin organization, and expression levels of Lifeact need to be kept at low levels to avoid artificial effects.

P1029/B30

Dynamic PIKfyve-containing puncta regulate lamellipodia dynamics.

H. Karabürk[1], G. Luo[1], R. Benyair[1], P. Rivero-Rios[1], G. Kreider-Letterman[2], R. Garcia-Mata[2], L. S. Weisman[1];
[1]University of Michigan, Ann Arbor, MI, [2]University of Toledo, Toledo, OH

Cell migration is an essential phenomenon in diverse biological processes including embryonic development, immune responses, and tissue repair. This directed movement of cells is controlled by chemical cues and signaling pathways. The force generated by actin polymerization within the cells drives cell migration by projecting membrane protrusions, including lamellipodia, at the leading edge of cells. Among those protrusions, lamellipodia are the major driver of cell migration in various cell types and are controlled by actin dynamics. Importantly, we and others recently reported that the PI3P 5-kinase, PIKfyve, may also plays a role in cell migration. This result was surprising because PIKfyve was

best known for roles in the regulation of lysosomes, and in autophagic degradation. We showed that PIKfyve contributes to cell migration in part via an unexpected role in recycling β1-integrin from early endosomes to the plasma membrane. Our new unpublished data suggests that PIKfyve has additional critical roles in cell migration via the regulation of actin dynamics in lamellipodia. Notably, we observed that acute chemical inhibition of PIKfyve for 5-10 minutes impaired lamellipodia dynamics and reduced the levels of F-actin in cell protrusions. Using TIRF live-cell imaging, we showed that a pool of dynamic PIKfyve-containing puncta localize to the cell protrusions. To gain insight into downstream targets of PIKfyve, we performed proximity-dependent biotinylation and we are currently pursuing targets related to actin dynamics. Together our new studies expand the known roles for PIKfyve, which was formerly thought to solely govern endo/lysosomes, and importantly suggest that PIKfyve is a regulator of multiple F-actin structures and cell migration.

P1030/B31

The Roles of Arp2/3 Complex Nucleation Promoting Factors in the C. elegans Single-Cell Embryo.

L. Leeming, S. E. Yde, R. S. Kadzik, E. Munro, D. R. Kovar;
University of Chicago, Chicago, IL

The Arp2/3 complex is the only known actin assembly factor capable of forming branched filamentous actin (F-actin) networks. In the single-cell C. elegans embryo, Arp2/3 complex concurrently assembles multiple branched F-actin networks at the cell cortex: punctate networks called mini-comets, and the bases of filopodia. Mini-comets are entirely branched, while filopodia are hybrid structures with a branched base and a bundled tip elongated by the formin Cyk-1. To investigate whether Arp2/3 complex was differentially activated in filopodia bases and mini-comets, we sought to characterize the activity of Wiskott-Aldrich Syndrome Protein (WASP) and WASP family verprolin-homologous protein (WAVE) complex, two well known branched actin nucleation promoting factors (NPFs) which are expressed at the single-cell stage.

Using two and three color live cell TIRF microscopy, we observed that both WAVE and WASP are enriched at the anterior cortex, but with distinct localization patterns. While WASP localizes to all cortical branched F-actin networks, WAVE colocalizes with Cyk-1 to the tips of filopodia rather than the branched base. We also observed that RNAi mediated knockdown of WAVE significantly reduced filopodia assembly. Filopodia numbers were not reduced in WASP mutants or when Arp2/3 complex is knocked down, however both perturbations result in altered filopodial dynamics.

These results suggest that WAVE and WASP are working together, and with other actin binding proteins, to assemble filopodia with the proper dynamics and spatiotemporal localization. However, whereas WASP is likely acting to promote Arp2/3 mediated branched actin assembly in the base, WAVE is likely acting in filopodia initiation independently of Arp2/3 complex. Specifically, WAVE could be acting directly on actin filaments as a processive elongator or collaboratively with another actin assembly factor at filopodia tips.

P1031/B32

System Integration through Contractility Kits Mediate Cell Shapes Change Processes.

M. C. Jacob;
Johns Hopkins School of Medicine, Baltimore, MD

Our comprehension of biological systems often rests within isolated domains, for example, with the cytoskeleton conventionally recognized for its structural and contractile roles in cellular processes, like

cytokinesis, motility, and morphology. In *Dictyostelium discoideum*, the presence of pre-assembled contractility kits (CKs) in the cytoplasm highlights a nuanced aspect of cytoskeletal function. These CKs, comprising various cytoskeletal components, respond to mechanical stress by accumulating along the cortex. Recent investigations in our lab, including genetic suppressor selections and proteomic analyses, have illuminated the cytoskeleton's involvement in intersystem crosstalk. Two proteins, RNA-binding protein 1 (RNP1A) and methylmalonate-semialdehyde dehydrogenase (mmsdh), emerged as potential mediators of intersystem communication with the cytoskeleton. RNP1A contributes to cell mechanics and mechanoresponsiveness while also binding to several mRNAs that encode proteins involved in macropinocytosis. Manipulation of RNP1A levels alters macropinocytosis activity, highlighting its role in this process. Proteomic studies reveal RNP1A's interaction with cortexillin I and IQGAP1, key CK components, indicating its involvement in cytoskeletal dynamics. On the other hand, mmsdh, an enzyme involved in valine catabolism, exhibits localization within mitochondria and the cytoplasm. In addition to its metabolic functions, mmsdh's role in cellular processes remains poorly understood. Notably, overexpression of mmsdh rescues cytokinetic defects caused by phosphomimetic non-muscle myosin II mutations. Proteomic analyses identify mmsdh's interaction with cortexillin I and myosin II, further implicating its involvement in CK dynamics. Knocking down mmsdh expression introduced several phenotypes such as a slower growth rate, reduction in cell size, and aberrant cytokinetic behavior. Combining insights from both studies, we formulate two hypotheses: first, RNP1A orchestrates coordination between macropinocytosis and the cytoskeleton through interaction with CK components; second, mmsdh modulates cytoskeletal dynamics and metabolic function via the CK network.

P1032/B33

Determining how physiological profilin-actin concentrations affect G-actin partitioning between Arp2/3 complex- and formin-mediated F-actin network assembly in fission yeast.

A. McCambridge, C. Suarez, R. Kadzik, D. R. Kovar;
University of Chicago, Chicago, IL

Actin-binding proteins (ABPs) regulate filamentous actin (F-actin) elongation, architecture, and stability, which facilitate the formation of distinct F-actin networks for diverse fundamental cellular processes. The actin assembly factors formin and Arp2/3 complex compete for a limiting globular actin (G-actin) pool, which temporally controls F-actin network assembly by incorporating G-actin into different F-actin networks. The small ABP profilin regulates G-actin competition by favoring G-actin partitioning into formin- over Arp2/3 complex-mediated networks. Profilin binds most of the G-actin pool to maintain an unpolymerized G-actin population and partitions G-actin in fission yeast to accelerate formin-mediated F-actin elongation but inhibits Arp2/3 complex-mediated F-actin branching. I am using in vitro spontaneous actin assembly assays with fission yeast formins, Arp2/3 complex, and profilin-actin visualized with Total Internal Reflection Fluorescence Microscopy (TIRFM) to determine how profilin affects competition between Arp2/3 complex and formin for G-actin as well as how physiological profilin-actin concentrations affect formin-mediated F-actin elongation and Arp2/3 complex-mediated F-actin branching. Understanding whether profilin affects G-actin partitioning at physiological concentrations in vitro and the profilin’s Arp2/3 complex inhibitory mechanism is critical to understanding how profilin can exert spatial-temporal control of F-actin network assembly.

P1033/B34

Emergent Mechanisms of Load Adaptation in Endocytic Actin Networks From Force-Attenuated Capping.

A. Raghunathan, M. Akamatsu;
University of Washington, Seattle, WA.

Force production by actin polymerization is crucial for several membrane-deforming cellular processes. These forces can be explained by an elastic Brownian Ratchet mechanism: actin filaments growing against a load fluctuate due to thermal motion, opening gaps for monomer addition, and restoration of filament position pushes the load forward. Critically, this implies that the rate of filament capping decreases with antagonistic force experienced by the filament (force-attenuated capping), which governs the load adaptation of in vitro actin networks by increasing filament density (Li et al., 2022). However, the mechanism by which such single filament load-dependent rates influence load adaptation by assemblies of actin networks participating in cellular processes remains unexplored. To address this question, we extended our experimentally-grounded agent-based model of actin networks at sites of clathrin mediated endocytosis (Akamatsu et al., 2020) to include force-attenuated capping, constrained by published in vitro measurements. We hypothesized that force-attenuated capping would allow endocytic actin filaments to adapt to increased loads by growing longer and thereby produce more force. Indeed, we found that implementing force-attenuated capping in the model allowed actin networks to produce ~2x more cumulative force and further internalize simulated endocytic pits. To investigate the mechanism by which force-attenuated capping increased endocytic pit internalization, we quantified filament lengths and numbers. Surprisingly, force-attenuated capping led to only a small (<10%) increase in average actin filament lengths. This is likely because only a small fraction of filaments experienced force, a previously unappreciated feature of such actin networks. In contrast, force-attenuated capping led to a ~40% increase in the total number of actin filaments, indicating an emergent consequence of increased filament nucleation. Importantly, under increasing load from membrane tension, force-attenuated capping led to further increased force production and more nucleated actin filaments. Taken together, these results identify a novel form of load adaptation, by which attenuating the capping rate of a small number of actin filaments leads to disproportionately increased filament nucleation, to increase force production against increasing resistance in endocytosis.

P1034/B35

Low intensity vibration improves apparent cell density and F-actin modeling in a 3D micro pellet model of chondrogenesis.

N. R. S. Silva, G. Uzer;
Boise State University, Boise, ID

Loss of mechanical loads during the transition from cartilage to bone, as it happens on developmental dysplasia of the hip, are the leading cause of early-onset hip osteoarthritis in individuals under the age of 50 among Americans. Previous studies have shown that low-intensity vibrations (LIV) enhance bone formation in both animals and in vitro models, as it upregulates pathways involved on chondrogenic and osteogenic responses, facilitating tissue differentiation. However, no studies have specifically explored the role of LIV in regulating chondrogenic tissue development. This study aims to address this gap by investigating the effects of LIV on the chondrogenic differentiation of MSCs using a hydrogel model, focusing on LIV's potential to enhance differentiation. To investigate, we encapsulated micro-pellets of MSCs ($6x10^4$ cells/pellet) in a hydrogel, with approximately $9.6x10^6$ cells/hydrogel. The samples were

divided into four groups: +LIV and -LIV, both with and without chondrogenic medium subgroups. For the +LIV groups, the gels were vibrated (25Hz, .5g) 4x/day for 14 days. Upon harvesting, we assessed cell viability, cell and actin density, and glycosaminoglycan (GAG) production using standard IHC staining protocols. Our results indicate that combining LIV with chondrogenic medium improves cell viability (+41%), cell density (+14%), and F-actin density (+74%). When pellets from the non-chondrogenic medium group were exposed to LIV, even greater increases were observed: +70% in cell viability, +103% in cell density and +1096% in fiber density. We did not observe significant changes in GAG with LIV. These results strengthen the idea that mechanical strains provided by LIV might help maintain cytoskeletal integrity, which in turn supports cell survival and differentiation. This study highlights the potential of LIV to enhance chondrogenic differentiation and improve protocol viability using MSCs. It also opens new avenues for exploring how mechanical strains influence the regulation of the cytoskeleton, and key markers in both chondrogenic and osteogenic pathways. As β-catenin (1) plays a pivotal role in chondrocyte development (maturation, hypertrophy, organization, and plasticity) and the formation of primary ossification centers, and (2) has shown to be responsive (upregulated) by LIV in the past, we are currently investigating the role of LIV in regulating β-catenin in chondrogenic tissues.

P1035/B36

Bequeathing a fair inheritance:How an unconventional fungus divides its growth potential among multiple daughter cells.

A. C. E. Wirshing[1], R. A. Matilla[2], D. Odde[2], D. J. Lew[1];
[1]MIT, Cambridge, MA, [2]University of Minnesota, Minneapolis, MN

Tractable yeast model systems have propelled fundamental discoveries in cell biology, but they do not encompass the full diversity of fungal cell biology. Unlike well-studied yeasts that produce only a single daughter cell (bud) per cell cycle, the unconventional yeast, *Aureobasidium pullulans*, can produce multiple buds simultaneously in a single cell cycle. This growth strategy raises the question of how growth potential is distributed between a mother's multiple offspring. In *S. cerevisiae*, post-Golgi secretory vesicles produced in the mother carry new cell membrane and cell wall components to the bud along polarized actin cables. We find that *A. pullulans* similarly produces secretory vesicles in the mother cell. These vesicles appear to be delivered to each bud at similar rates, because sister buds growing from the same mother grow at similar rates (CV = 0.15). Interestingly, buds grow at similar rates even in cases where the bud sites are asymmetrically distributed around the mother. Modeling suggests that in such situations, clustered buds would compete for local vesicles while distant buds would be able to access vesicles from a larger mother volume. This raises the question of how vesicle traffic to each bud is equalized. *A. pullulans* formins localize to each bud tip and build a polarized and dynamic network of actin cables that extends along the mother cell cortex. However, the network does not exhibit obvious compensation that could explain equal vesicle delivery to buds in differing geometries. Additionally, *A. pullulans* develops an interphase microtubule network that extends throughout the volume of the mother cell with the plus ends of microtubules oriented into each bud. By tracking processive vesicle movements in live cells, we find that both F-actin and microtubules can mediate vesicle transport. Based on these findings, we speculate that each bud's actin network catches and delivers vesicles from the bud-proximal mother volume, while microtubule networks may help to distribute vesicles throughout the mother so that each bud's actin network can access similar numbers of vesicles.

P1036/B37

Actin Modification by Mical and SelR Is Necessary for Actomyosin Ring Dynamics and Efficient Cell Wound Repair.

M. Nakamura, C. Aarrestad, S. M. Parkhurst;
Fred Hutchinson Cancer Center, Seattle, WA

Individual cells within tissues and organs are subjected to damage caused by daily wear-and-tear and environmental/physiological stresses. To survive this damage and remain functional, cells have a robust repair mechanism comprised of rapid membrane resealing/remodeling and dynamic cytoskeletal repair at the cell cortex that are initiated by calcium influx. Actomyosin ring contraction at the wound edge is one mechanism that can generate the physical force needed for wound closure. However, little is known about how the actomyosin ring assembles upon wounding, is dynamically organized, or disassembles after wound closure. Using the *Drosophila* cell wound repair model, we recently found that Rab35 is recruited to wounds and its RNAi knockdown disrupts actomyosin ring assembly and disassembly and exhibits slow wound closure. Rab35, a member of Rab family GTPases, regulates membrane trafficking and cytoskeleton dynamics in many cellular processes. In particular, Rab35 regulates Mical, a redox enzyme that binds to F-actin and promotes F-actin clearance during cytokinetic abscission. SelR is a methionine sulfoxide reduction enzyme that works as a counterpart to Mical. We find that both Mical and SelR are recruited to cell wounds. We find that both Mical and SelR RNAi knockdowns exhibit disrupted actomyosin ring organization and dynamics at the wound edge. To further examine the redox reaction of actin during cell wound repair, we generated a transgenic line having a point mutation on Methionine 44 that is known to be oxidized by Mical. Consistent with Mical knockdown, the M44 point mutation impairs actomyosin ring formation leading to inefficient cell wound repair. Our results suggest that actin modifications play a mechanistic role in the assembly and disassembly of the actomyosin ring, which is necessary for efficient cell wound repair.

P1037/B38

Identification of TPD52L2 as a Regulator of Actin Dynamics and Cellular Mechanoadaptability.

A. Plesche[1], A. Parizadeh[1], F. Weber[2], N. Parizadeh[1], S. Geley[1], D. Fankhauser[1], V. Reiterer-Farhan[1], F. Baschieri[1], B. Plochberger[2], H. Farhan[1];
[1]Medical University of Innsbruck, Innsbruck, Austria, [2]University of Applied Sciences Upper Austria, Linz, Austria

Tumor protein D52 like 2 (TPD52L2) is part of the TPD52 protein family and is known to be overexpressed in a variety of malignant diseases. This includes breast cancer, where its overexpression correlates with a reduced overall survival. Recently, TPD52L2 was reported to be associated with intracellular nanovesicles, indicating its involvement in various membrane trafficking pathways. Our study unravels the role of TPD52L2 as a regulator of the actin cytoskeleton in breast cancer cells. Depletion of TPD52L2 results in a strong reduction of stress fibers, altered dynamics of actin protrusions and finally a thickening of the cortical actin layer. Mechanistically, we demonstrate that TPD52L2 interacts with Cullin-5, a central component of the Cullin-RING ubiquitin ligase (CRL) complex. Cullin-5 functions as a scaffold for the recruitment of Rab40 family members, which act as substrate adaptors for the ubiquitination and degradation of cytoskeletal proteins. The actin phenotype induced by silencing of TPD52L2 was partially rescued by co-depletion of Rab40c. We are currently testing a model wherein TPD52L2 regulates Rab40c-mediated degradation of actin regulators. As a result of the increased cortical actin in TPD52L2 depleted cells, aberrations of the mechanical properties were observed. Atomic force